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Seed Morphology of Three Neotropical Orchid Species of the *Lycaste* Genus

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Abstract: Neotropical orchids are vulnerable to extinction due to overharvesting, habitat destruction and climate change. However, a basic understanding of orchid seed biology to support conservation efforts is still lacking for most species. Seed morphology is linked to plant adaptation and evolution, influencing seed dispersal, dormancy, longevity, and germination, which are valuable traits for conservation. In this study, we characterized and compared the morphological traits of seed capsules (size, shape, and colour) and seeds (seed and embryo shape and size and internal airspace volume) for three epiphytic Neotropical orchid species of the genus *Lycaste* native to Guatemala: *L. cochleata*, *L. lasioglossa*, and *L. virginalis*. The three species show qualitative similarities in seed capsule colour and appearance and in seed morphology (i.e., scobiform oval-shaped seeds and prolate-spheroid embryos). All species have small-sized seeds (length of *L. cochleata*: 210 µm, *L. lasioglossa*: 230 µm, and *L. virginalis*: 260 µm), with proportionally large embryos (length of *L. cochleata*: 140 µm, *L. lasioglossa*: 120 µm, and *L. virginalis*: 150 µm) and an internal air-space volume that occupies less than half of the seed (*L. cochleata*: 17%, *L. lasioglossa*: 42%, and *L. virginalis*: 30%). This finding is consistent with previous reports for other epiphytic orchid species, which typically have lower air volumes than terrestrial orchids. These differences are likely a result of evolutionary changes associated with different habits and may influence seed dispersal. We also found some significant differences in seed morphology between the studied species, but their taxonomic, biological, and ecological relevance remain to be elucidated. More comparative studies, including on other *Lycaste* species with different habits, are needed to explore relationships between seed morphology, taxonomy, biology, and ecology in this genus to support its conservation.

Keywords: adaptation; airspace; conservation; embryo; epiphytic; morphometry

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1. Introduction

Worldwide, the greatest diversity of orchids can be found in the Neotropics, particularly in cloud forests, which provide favourable habitats for orchid growth [1,2]. Neotropical orchids have attractive floral displays and are extremely desired for horticulture, medicine, and food [3,4]. However, they have complex biological and ecological features, with most of them being epiphytic, relying on animal pollination for reproduction and fungal symbionts for germination, alongside having unique physiological adaptations to tropical climates [5,6]. Therefore, unsustainable harvesting and the destruction of their natural habitat, combined with climate change, make these orchids particularly vulnerable to extinction. For this reason, it is urgent to develop both in situ as well as ex situ conservation programs to preserve them. One of the limitations in developing these programs is the lack of information on the basic ecology and biology for most Neotropical orchid species.

Guatemala is a biodiversity hotspot, home to over 1400 identified orchid species [7,8]. Among these, *Lycaste* species have high ethnobotanical significance since some species have traditionally been used by indigenous peoples dating back to the Mayan civilization [9]. The genus *Lycaste* Lindl. Belongs to the subfamily Epidendroideae and the tribe Maxillarieae and consists of about thirty-six (36) accepted species with epiphytic, terrestrial, or lithophytic habits [10,11]. These species are naturally distributed throughout the south of Mexico, Central America, and the north of South America [12]. They are characterized by short and thickened pseudobulbs, with flowers that are generally large, very attractive, often with a pleasant, sweet scent, and which develop three sepals, two petals, and a third sepal called labellum [12]. Many species of *Lycaste* are highly desirable in horticulture due to their appearance and colour variations, which make them more susceptible to overharvest.

Three *Lycaste* species were selected to explore their seed capsules and morphology: *L. virginalis* (Scheidw.) Linden, *L. lasioglossa* Rchb.f., and *L. cochleata* Lindl. (synonym of *Selbyana cochleata* (Lindl.) Archila). *Lycaste virginalis f. alba* (B.S. Williams) Archila & Chiron, commonly known as "Monja Blanca" (white nun), has been the National Flower of Guatemala since 1934. It holds high cultural and patriotic values, and it is often displayed at national celebrations. However, *L. virginalis* is at a high and increased risk of extinction [13]. *Lycaste cochleata* is very rare and has a limited distribution from southeastern Mexico to Nicaragua [12,13]. *Lycaste lasioglossa* also is very rare, and is distinguished from all other *Lycaste* species found in Guatemala by its densely pubescent labellum; this species is not at risk of extinction yet, but its trade is regulated [12–14].

Seeds are essential for the species' adaptation, regeneration, distribution, and persistence, playing a significant role in orchid conservation strategies [15,16]. Seed morphology is associated with important processes such as seed dispersal, dormancy, germination, and establishment, which are all relevant to seed conservation [15]. Seed traits are thought to be more conservative than floral and vegetative characteristics, and consequently they are also valuable indicators of the taxonomy, phylogenetics, and phytogeographic distribution of orchid species [16,17].

Most orchid seeds are small (dust-like), with a low weight. A single capsule (the reproductive unit) can produce thousands of seeds [17]. Due to their minuscule size, seed characteristics remain undescribed for many orchid species. Orchid seeds typically consist of a seed coat that incorporates an embryo and an internal air space volume (between the seed coat and embryo), lacking an endosperm [17].

The relationship between seed and embryo volumes determines the proportion of air space within the seeds. The internal air space of the seeds varies depending on the species [15]. Previous studies suggest that epiphytic orchids typically have lower airspace volumes (~30% on average), while terrestrial species have larger air volumes (~60% on average) [16,18]. These differences are likely to impact seed dispersal, germination, dormancy, and establishment [15–19].

Understanding seed traits is essential to inform conservation efforts. Therefore, species-specific studies to characterize seed morphological traits (including the airspace volume) are useful to identify the most suitable conservation approaches for each taxon [15]. Therefore, this study aimed to characterize and compare the seed capsule and seed morphological traits of three tropical rare and threatened epiphytic species from Guatemala: *L. cochleata*, *L. lasioglossa*, and *L. virginalis*. To this end, we measured qualitative traits (seed capsule colour and shape, seed and embryo shape) and quantitative traits (capsule size, seed size, embryo size, airspace volume), and calculated the relationships between quantitative traits to assess the relative occupancy of the embryo and air space within the seeds.

2. Materials and Methods

2.1. Seed Material

Lycaste cochleata, *L. lasioglossa*, and *L. virginalis* (Figure 1) were grown at the Experimental Orchid Station Farm of the Archila family located in the tropical forest of Guatemala (temperatures ranging from 12.5 °C to 27.8 °C, relative humidity from 76% to 91%, latitude

15°29′0″ N, longitude 90°22′0″ W, and an altitude of 1316 m a.s.l.) [20]. The flowers were self-pollinated by hand and the seed capsules harvested when mature (i.e., capsules that completed development and were fully grown) and about to naturally dehisce (i.e., gape or burst open). After harvesting, the seed capsules were placed in different plastic containers with silica gel to absorb humidity and tissue paper on the top and bottom to minimize movement, surrounded by a cold shipping package that consisted of a standard duration cooling system of 96 h at a constant 2 to 5 °C (provided by a Guatemalan supplier, SM Soluciones S.A.) and sent by courier to Massey University Turitea Campus in New Zealand (1–2 weeks). Upon arrival, the seed capsules were placed in plastic containers to dry in a controlled environment room operating at 20 °C and 55% relative humidity (RH). The seed capsules were allowed to open naturally under those conditions, and exposed seeds were then collected and stored in glass vessels under the same conditions (20 °C and 55% RH) before being assessed.



Figure 1. Flowers of (**a**) *Lycaste cochleata*, (**b**) *L. lasioglossa*, and (**c**) *L. virginalis* (images by Fredy Archila Morales).

2.2. Seed Capsule Assessment

To observe the morphological variation of the seed capsules, we assessed ten capsules from different individual plants per species (upon arrival in NZ).Each capsule was treated as a replicate. Each capsule's length, top diameter, central diameter, and bottom diameter were measured using a vernier calliper. The measurement consisted of placing the seed capsules longitudinally (for length) and transversely (for diameter) between the two vernier scales. In addition, we visually evaluated and compared the capsule shape, particularly the number of ribs per capsule and the colour of the seed capsules, which was examined using the Pantone Colour Matching System [21].

2.3. Seed and Embryo Morphology

Prior to undertaking the measurements, seed samples of five different capsules per species were stained with tetrazolium chloride [22]. We used this staining method for ease of observation under the microscope, and recorded seed viability data (indicated by red staining the embryo), which will be reported as part of a separate study. The tetrazolium test was performed following the method employed by Diantina [23]. Firstly, seed samples were placed in plastic vials with a sucrose solution at 10% (w/v) in a room at 20 °C for twenty-four hours. Afterwards, the sucrose solution was replaced with 2,3,5-triphenyl tetrazolium chloride at 1% (w/v), and then the seeds were incubated in a dark incubator at 40 °C for another twenty-four hours. Lastly, ten red-stained seeds per capsule (50 seeds per species) were measured for the length and width of both the seed and the embryo under a binocular microscope Olympus SZX7 at magnification 5.6×.

The seed volume (SV), embryo volume (EV), and percentage of air space within the seed (ASV) were estimated using the formulas established by Arditti and Ghani [24] for seeds with prolate spheroid embryos:

$$SV = 2\left(\left(SW/2\right)^2 * \left(SL/2\right) * 1.047\right)$$

$$EV = {}^{4}/_{3}\ \pi * ({}^{EL}/_{2}) * ({}^{EW}/_{2})^{2}$$

$$ASV\ (\%) = \left(\frac{SV - EV}{SV}\right) * 100$$

where SW = seed width, SL = seed length, EW = embryo width, and EL = embryo length.

The seed length and seed width (SL/SW), embryo length and embryo width (EL/EW), and seed volume with embryo volume (SV/EV) relationship were assessed to confirm the relative occupancy of the embryo and air space within the seeds.

2.4. Data Analysis

The Shapiro–Wilk test was used to confirm that data met the premises of normality ($p > 0.05$). A general linear model (GLM) was performed to explore differences in the quantitative seed coat and seed traits among species. Afterwards, means were compared with a post hoc Tukey's test (significance 5%), using the software 'IBM SPSS Statistics' version 28.0.1.1. [25]. In addition, the relationships of the different seed morphological parameters studied, SL/SW, EL/EW, and SV/EV, were analysed as described above.

3. Results

3.1. Seed Capsule Morphology

The seed capsules of the three species had six longitudinal ribs, as is common for other orchid species [26,27]. The seed capsules were considered ripe when their colour was yellowish green (Pantone 3604-382) (Figure 2). On drying, the capsules turned brown with some small green–yellow spots (Pantone 1255-1265-582-457) (Figure 2).

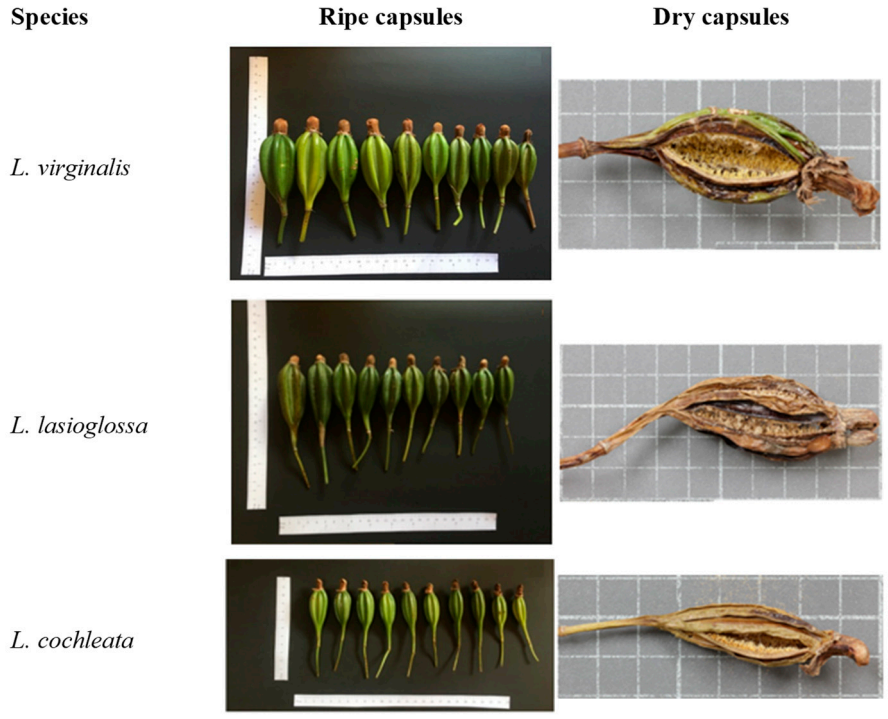


Figure 2. Variability of ripe and dry seed capsules of *Lycaste virginalis*, *L. lasioglossa*, and *L. cochleata*. The scale in the left pictures is a 30 cm ruler, and in the right ones each square is 1 cm^2.

Table 1 shows the results of a general linear model (GLM), which revealed that *L. cochleata* capsules were significantly different in mean length (F (2, 27) = 13.28, $p = 0.0$) and in top diameter (F (2, 27) = 47.88, $p = 0.0$) compared with *L. lasioglossa* and *L. virginalis*. For the central diameter, there were no significant differences in means across the three

species (F (2, 27) = 1.5, $p = 0.24$). For the bottom diameter, there was a statistically significant difference in the mean between all three species (F (2, 27) = 28.5, $p = 0.0$), with *L. virginalis* having the largest diameter and *L. cochleata* the smallest.

Table 1. Variability in seed capsule length and diameter (Ø) of the three different *Lycaste* orchid species.

Species	Capsule Measurement (cm)			
	Length	Ø Top	Ø Central	Ø Bottom
L. cochleata	7.90 ± 0.74^{b}	1.0 ± 0.00^{b}	$2.1 \pm 2.13^{n.s.}$	1.0 ± 0.00^{c}
L. lasioglossa	9.80 ± 1.03^{a}	2.1 ± 0.32^{a}	$2.9 \pm 0.32^{n.s.}$	1.6 ± 0.52^{b}
L. virginalis	10.3 ± 1.42^{a}	2.2 ± 0.42^{a}	$3.0 \pm 0.47^{n.s.}$	2.0 ± 0.00^{a}

Each individual value (mean ± SD) is followed by letters that indicate significant differences when present (Tukey test, $p < 0.05$). n.s. indicates that no significant differences were found for this trait.

3.2. Seed Morphology

Lycaste has fine, yellowish, sand-like seeds. The colour intensity varies slightly between each species (*L. cochleata*: Pantone 393, *L. lasioglossa*: Pantone 615, and *L. virginalis*: Pantone 459) (Figure 3) [21]. Under the microscope, it was possible to distinguish the seed coat and embryo. The three species have scobiform oval-shaped seeds and prolate spheroid embryos, as defined by [15,16] (Figure 3).

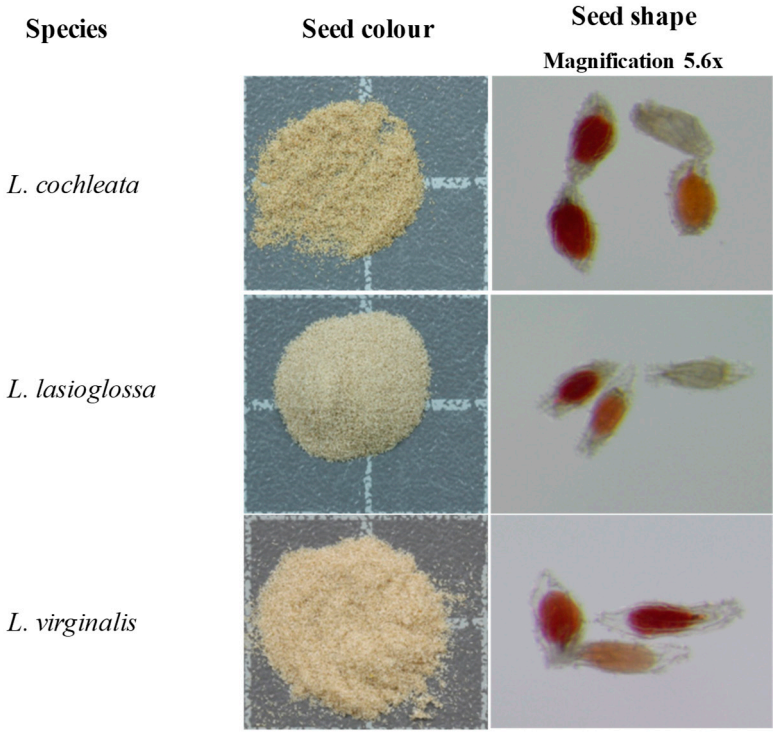


Figure 3. *Lycaste* seeds without (left photographs) and with 5.6× magnification (right photographs). The seeds enlarged at 5.6× are stained with 2,3,5-Tetrazolium Chloride (TTC) for ease of visualization under the microscope. Each square on the left side is 1 cm^2.

GLM analysis revealed that there was a statistically significant difference in the seed dimensions (SL = seed length, SW = seed width, and SV = seed volume), and significant differences within the embryo dimensions (EL = embryo length, EW = embryo width, and EV = embryo volume) between the three species (Table 2). The data showed that the mean air-space percentage differed significantly between the three species (F (2, 147) = 105.62, $p = 0.0$), with *L. lasioglossa* having the highest seed air space volume and *L. cochleata* the lowest, but all were less than 50% of the total seed volume. Likewise, the post hoc comparison

using Tukey's test after GLM found that the mean value of the seed morphological traits was significantly different between the three species, but this differed for individual traits, with *L. virginalis* having the longest seed but an embryo equal in length to *L. cochleata.* The largest embryo volume was found in *L. cochleata*.

Table 2. Seed and embryo dimensions for three *Lycaste* species.

Seed Dimensions				
Species	**Length (mm)**	**Width (mm)**	**Volume (mm^3)**	
L. cochleata	0.21 ± 0.02 ^{c}	0.11 ± 0.01 ^{a}	0.00063 ± 0.0002 ^{a}	
L. lasioglossa	0.23 ± 0.03 ^{b}	0.09 ± 0.01 ^{b}	0.00051 ± 0.0001 ^{b}	
L. virginalis	0.26 ± 0.02 ^{a}	0.09 ± 0.01 ^{b}	0.00060 ± 0.0002 ^{a}	
Embryo Dimensions				
Species	**Length (mm)**	**Width (mm)**	**Volume (mm^3)**	**Air-space % in the Seed**
L. cochleata	0.14 ± 0.01 ^{a}	0.082 ± 0.01 ^{a}	0.00051 ± 0.00011 ^{a}	17.4 ± 8.0 ^{c}
L. lasioglossa	0.12 ± 0.01 ^{b}	0.066 ± 0.01 ^{c}	0.00029 ± 0.0007 ^{c}	42.3 ± 8.1 ^{a}
L. virginalis	0.15 ± 0.01 ^{a}	0.073 ± 0.01 ^{b}	0.00041 ± 0.00009 ^{b}	29.7 ± 9.5 ^{b}

Each individual value (mean ± SD) is followed by letters that indicate significant differences when different (Tukey test, $p < 0.05$).

Table 3 shows comparison of the relationship between some of the morphological parameters evaluated, i.e., seed length (SL) with seed width (SW); the ratio of embryo length (EL) with embryo width (EW); and seed volume (SV) with embryo volume (EV). The SL/SW and SV/EV differed across all three species.

Table 3. Relationship between seed length and seed width (SL/SW), embryo length and embryo width (EL/EW), and seed volume with embryo volume (SV/EV) of three *Lycaste* species.

Species	**SL/SW**	**EL/EW**	**SV/EV**
L. cochleata	1.98 ± 0.26 ^{c}	1.78 ± 0.24 ^{b}	1.22 ± 0.13 ^{c}
L. lasioglossa	0.57 ± 0.37 ^{b}	1.86 ± 0.19 ^{b}	1.77 ± 0.25 ^{a}
L. virginalis	2.75 ± 0.38 ^{a}	2.04 ± 0.26 ^{a}	1.45 ± 0.21 ^{b}

Different letters indicate significant differences ($p < 0.05$) between species for each trait (mean ± SD) after a Tukey post hoc test.

4. Discussion

4.1. Seed Capsule Morphology

In this study, we characterized the seed capsule morphology of three tropical epiphytic species of the genus *Lycaste*. The seed capsule morphological assessment was based on the capsule's length, diameter (i.e., top, middle, bottom), and colour. Our results show that the seed capsules of the three species are very similar in appearance and colour (Figure 2). Other studies have reported similarities in seed capsule shape and colour for other *Lycaste* species [12,17,27].

Despite qualitative similarities, the capsules' quantitative traits varied between species, with *L. cochleata* having comparatively smaller capsules than the other species (Table 1). We also observed some intraspecific variation in capsule size (Figure 2). These differences could reflect species-specific adaptations to their natural environment [23,28] related to capsule position, or could be a result of differences in resource availability to plants when developing the capsules (the seed capsules were produced in the same location at the same time, and the collected capsules were positioned in the same location on different plants; however, the plants grew outdoors, where many factors were not controlled). Other factors, such as intra-plant competition, may also have contributed to the observed differences (i.e., competition between individuals of the same species for resources, edge effects). More research over several production seasons and under controlled conditions is required to elucidate the reasons behind inter- and intraspecific variability.

4.2. Seed Morphology

Like for the seed capsules, multiple traits are shared by the seeds of the three *Lycaste* species, e.g., they seeds are small, oval-shaped, scobiform, and have comparatively large embryos and lower air volumes. Similar traits (small ~300 µm, scobiform seeds) have been previously reported for *Lycaste skinneri* by [17], who also described testa seed characteristics (not explored here).

Orchid seed sizes can be categorized based on their length, from very small (100–200 µm), small (200–500 µm), medium (500–900 µm), and large (900–2000 µm), to very large (2000–6000 µm) [17]. Based on this classification, *L. cochleata*, *L. lasioglossa*, and *L. virginalis* are all considered species with small seeds (210, 230, and 260 µm, respectively). During the evolutionary process, morphological differences occurred in orchid seeds as an adaptation to different habitats (e.g., epiphytic vs. terrestrial), climates (e.g., tropical vs. temperate), and modes of dispersal (e.g., anemochory, zoochory, hydrochory) [16]. The three *Lycaste* species studied are tropical epiphytes, and more information is needed about their dispersal mechanisms.

Our results show that the three *Lycaste* species have low air volumes, which is consistent with other epiphytic orchids [15,16,18]. Epiphytic orchids (e.g., many *Dendrobium* spp.) typically have smaller air spaces than their terrestrial counterparts (e.g., *Paphiopedilum)* [15]. It has been suggested that a larger airspace was an advantage for ancestral terrestrial orchids; it causes higher buoyancy and floatability, and thus wider distribution areas [15,16,18]. The shift in seed aerodynamic traits is likely to be related to their evolutionary changes in growth habit, from terrestrial to epiphytic, suggesting lower dispersibility in epiphytes [18].

We found the embryo dimensions to be similar to those reported for other tropical epiphytic species, e.g., *Dendrobium* spp. [29,30]. A comparative study between closely related terrestrial and epiphytic species of the *Liparis* genus in Japan found that epiphytic species had larger embryos and suggested that seeds with larger embryos are likely to be developmentally advanced and have the potential to germinate earlier, facilitating seed establishment in epiphytic species which lack a soil substrate [18]. However, embryo size is highly variable among orchids [17], and other studies found no relationship between embryo size and habit for other genera [23,31]. More studies on *Lycaste* species with different habits are needed to explore whether there is a relationship between embryo size, habit, and germination in this genus.

Seeds with large embryos have also been reported as non-dormant, an adaptation that may allow them to germinate faster in tropical environments, as opposed to in temperate environments where seeds may require dormancy until certain environmental conditions are met to support germination [15,30]. In this study, seed dormancy was not evaluated but we hypothesize that seeds are non-dormant, given their natural distribution (tropical habitats). Further research is needed to validate whether *Lycaste*'s seed morphological traits are associated with a lack of dormancy, as predicted.

While many similarities exist, we also recorded some variability in the seed morphological traits across *Lycaste* species. Of particular interest are the differences in embryo volume and air space percentage, which differ significantly between species and could influence important processes such as seed dispersal, dormancy, germination, and establishment. *L. lasioglossa* has smaller seeds, smaller embryos, and a larger air volume than the other two species, but the biological and ecological implications of these differences remain to be elucidated.

We encourage further comparative studies including other *Lycaste* species to establish relationships between seed morphology and biological and ecological traits. The exploration of other morphological traits not investigated here (e.g., additional seed coat characters, ovary size, testa cell shape and dimensions, etc.) could also add valuable information for species comparisons. This is only a small contribution to the knowledge of Guatemalan (and Neotropical) orchid species, but more studies on seed morphology,

ecology, chemistry, and storage such as those conducted for other species, e.g., [23,30,32,33], are vital to ensure the conservation of Neotropical orchids for future generations.

5. Conclusions

Overall, our results show similarities in the qualitative traits of the seed capsules and the seeds of *Lycaste*. While similarities exist, we also report significant differences in the quantitative characteristics of the seed capsules and seed micromorphology of the three studied species. The implication of these differences for taxonomy, seed biology, and ecology remain to be elucidated.

Like other epiphytic orchids, *Lycaste* species have relatively small air volumes compared to terrestrial species, which is thought to be an evolutionary adaptation related to seed dispersal. Smaller air volumes are associated with less floatability and buoyancy, restricting seed dispersal, and probably contribute to the limited distribution of these species.

This characterization is of value to systematic botany because it contributes to our understanding of orchid seed micromorphology in three Neotropical species of the *Lycaste* genus. However, we only contributed to the study of a small proportion of the total number of species in the *Lycaste* genus (3 species out of 36); more comparative studies including other *Lycaste* species are needed to explore the relationships between seed morphology, taxonomy, biology, and ecology to inform conservation efforts.

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Conflicts of Interest: The authors declare no conflict of interest.

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